Clinical Trial Protocol

A Study to Evaluate the Therapeutic Effect and Safety of Metagenomics-Based Probiotics in Patients with Non-Alcoholic Fatty Liver Disease (NAFLD)

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Confidential

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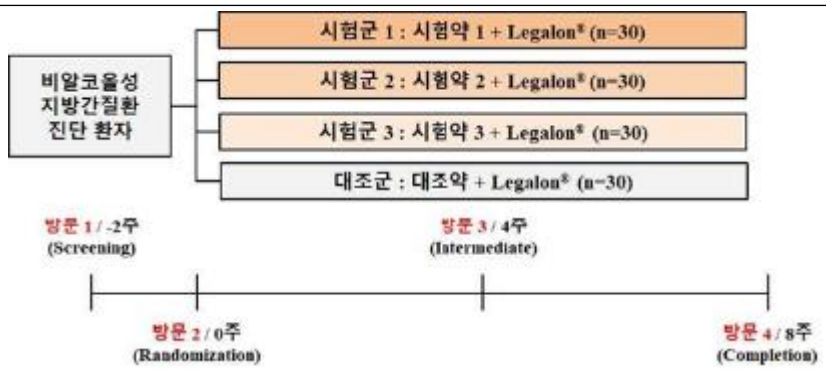
◎ **Summary**

Clinical Trial Title	A Study to Evaluate the Therapeutic Effect and Safety of Metagenomics-Based Probiotics in Patients with Non-Alcoholic Fatty Liver Disease (NAFLD)
Study Site:	Hallym University Chuncheon Sacred Heart Hospital 7 Sakju-ro, Chuncheon-si, Gangwon-do, Republic of Korea * Subject recruitment and study visits will be conducted at Hallym University Chuncheon Sacred Heart Hospital. Human-derived sample analysis will be performed by ChunLab, Inc.
Principal Investigator	Prof. Ki Tae Seok, Department of Gastroenterology, Hallym University Chuncheon Sacred Heart Hospital
Study Phase:	Investigator-Initiated Clinical Trial
Sponsor	CKD Bio Corporation
Study Period Study Period	From IRB approval date to April 30, 2022
Disease Target	Non-Alcoholic Fatty Liver Disease (NAFLD)
Study Objective	To evaluate the therapeutic effect of probiotics on improving non-alcoholic fatty liver disease in patients diagnosed with NAFLD.
Study Design	8-week, single-center, randomized, double-blind, placebo-controlled, parallel-group clinical trial
Investigational Products:	*- Capsule containing Pediococcus pentosaceus KID7 (9×10^9 CFU/day)* *- Capsule containing Lactobacillus helveticus CKDB001 (9×10^9 CFU/day)* *- Capsule containing Lactobacillus lactis CKDB001 (9×10^9 CFU/day)*
Control Product	Placebo (identical appearance, containing microcrystalline cellulose, without probiotic strains)
Inclusion Criteria	Inclusion Criteria Participants who meet all of the following criteria will be enrolled in the study: 1. Individuals who voluntarily agree to participate and provide written informed consent. 2. Male or female adults aged 20 years or older. 3. Patients diagnosed with non-alcoholic fatty liver disease (NAFLD). - Alcohol-related liver disease exclusion threshold: - Average daily alcohol consumption ≥ 60 g/day in the past year (≥ 30 g/day for women). - Reference examples: Soju 360 mL, 20% alcohol = 72 g of alcohol Beer 500 mL (330 mL for commercial bottles), 5% alcohol = 25 g (16.5 g) 4. Patients with elevated liver enzyme levels above the normal range. - Reference normal ranges: - AST, ALT: ≤ 40 U/L - ALP: 20–130 U/L - GOT: 0–30 U/L - GPT: 0–38 U/L - GGT: 10–62 U/L (male), 7–35 U/L (female)
Exclusion Criteria	Participants who meet any of the following conditions will be excluded: 1. Use of probiotics (e.g., lactic acid bacteria), prebiotics (e.g., dietary fiber, fructooligosaccharides), synbiotics, or fermented dairy products within the past 1 month. 2. Continuous use of antibiotics within the past 2 months, or individuals likely to require antibiotic therapy during the study period. Individuals who have continuously taken medications or health functional foods that may affect liver function within the past 1 month.

	3. Individuals who have participated in another clinical trial within the past 1 month 4. Participation in medical device trials is exempted from this criterion. 5. Individuals with any of the following conditions: Alcoholic liver disease, hereditary metabolic disorders, or autoimmune hepatitis Systemic inflammatory diseases or immune-mediated disorders, Hepatocellular carcinoma, Uncontrolled cardiopulmonary disease, Other significant systemic disorders involving the heart, lungs, hematologic system, or endocrine system. Individuals with a history of malignant tumors diagnosed within the past 5 years, Pregnant or breastfeeding women. Individuals with known hypersensitivity to the investigational product/placebo or any of their components, or those with a history of severe allergic reactions. Individuals deemed unsuitable for participation in the clinical trial at the investigator's discretion.
Sample Size:	Target enrollment is 120 subjects (30 subjects per group, accounting for 10% dropout).
Methods	 Treatment Group 1: Investigational Product 1 + Legalon® (n=30) Treatment Group 2: Investigational Product 2 + Legalon® (n=30) Treatment Group 3: Investigational Product 3 + Legalon® (n=30) Control Group: Control Treatment + Legalon® (n=30) • Visit 1 (Week –2): Screening • Visit 2 (Week 0): Randomization / Baseline • Visit 3 (Week 4): Intermediate Assessment • Visit 4 (Week 8): Completion / Final Assessment
END POINT	1) Primary Efficacy Endpoint: - Change in liver enzyme levels (AST, ALT) 2) Secondary Efficacy Endpoints: - Change in GGT, albumin - Lipid profile changes (cholesterol, triglycerides) - Inflammatory cytokine levels (TNF-α, IL-6) - BMI changes - Imaging changes (Ultrasound/CT, FibroScan) - MELD score - Child–Pugh score - Hepatic decompensation events (encephalopathy, ascites, variceal bleeding, SBP) - Hospitalization, mortality, liver transplantation - Stool habit changes - Quality of life - Gut microbiome composition 3) Human-derived specimen research and genetic testing For participants who provide separate written consent for human-derived specimen research and genetic testing, blood, serum, and stool samples will be collected. These specimens will be used independently from this clinical trial as research materials to investigate the prevention, diagnosis, and treatment of chronic liver diseases.

Analysis method	Primary Efficacy Endpoint Analysis After administering the investigational product or comparator for 8 weeks to the four randomized groups, changes in liver enzymes (AST, ALT) from baseline will be calculated for each group. Based on the results of normality and homogeneity of variance tests, a two-sample t-test or Mann–Whitney U test will be performed. Additional subgroup analyses may be conducted as needed. Secondary Efficacy Endpoint Analysis After 8 weeks of administration of the investigational product or comparator to the four randomized groups, the following parameters will be analyzed for changes from baseline using the same analytic approach: serum GGT and albumin levels; serum lipid profile (cholesterol, triglycerides); inflammatory cytokines (TNF-α, IL-6); BMI; imaging-based histologic parameters (abdominal ultrasound/CT, FibroScan); MELD score; Child–Pugh score; worsening of liver disease (hepatic encephalopathy, ascites, variceal bleeding, spontaneous bacterial peritonitis, liver-related hospitalization, death or liver transplantation, and all-cause mortality); bowel habits (frequency, stool volume, stool consistency and form, time required for defecation); quality of life (questionnaire-based); and gut microbiome composition. Additional subgroup analyses may also be performed. Stool Sample Analysis Stool samples will be obtained from participants who provide written informed consent for human biospecimen research. Microbiome analysis will be conducted by ChunLab, a specialized bioinformatics analysis company.
SAFETY ANALYSIS	Adverse events, vital signs, and clinical laboratory tests (blood and urine)

◎ **Study Timeline**

Procedure		**Screening**	**Baseline**	**Treatment**	**End of study**
Visit		**1**	**2**	**3**	**4**
Week		**-2**	**0**[1)]	**4**	**8**
Window period (day)		**±7**		**±7**	**±7**
Informed Consent of Study Participants		√			
Demographic Characteristics		√			
Physical Measurements		√	√	√	√
Vital Signs (Pulse, Blood Pressure, Body Temperature)		√	√	√	√
Medical and Surgical History Assessment		√			
Medication History Assessment		√	√	√	√
Alcohol and Smoking History Assessment		√	√	√	√
Screening Examinations (Blood and Urine Tests)	Blood Tests	√			
	Pregnancy Test	√			
	Viral Marker Testing	√			
Assessment of Inclusion and Exclusion Criteria		√			
Randomization			√		
Study Drug Administration			√	√	
Stool Sample Collection and Bowel Habit Assessment			√		√
Clinical Laboratory Tests (Blood)			√	√	√
Histopathological Examination			√	√	√
Assessment of Quality-of-Life Improvement			√		√
Liver biopsy			√		√
Monitoring and Assessment of Adverse Events		√	√	√	√

Visit 2 (Week 0) shall be conducted at least 2 weeks and within 3 weeks after Visit 1 (Week –2). (Visit 1 and Visit 2 may be conducted on the same day.)

Information on sex, date of birth, age, menstrual status, and duration of amenorrhea will be collected.

Body weight, height, and BMI will be measured. (Height will be measured only at Visit 1.)

Participants will visit after a 12-hour fast on the day prior to testing, and blood tests will be performed. (For participants with fatty liver disease, only clinically necessary items may be tested at the discretion of the principal investigator.)

① Blood biochemical tests: AST, ALT, ALP, GGT, albumin, total bilirubin, direct bilirubin, total protein, uric acid, BUN, creatinine, glucose, cholesterol, triglycerides (These tests may be omitted for participants who have health examination results within the past 4 weeks.)

A urine pregnancy test will be conducted for women of childbearing potential, except for those in confirmed menopause (amenorrhea ≥24 months).

Viral marker tests will include HBsAg, anti-HBs, and anti-HCV. (Testing will be performed when results within the past 5 years are unavailable and deemed clinically necessary by the investigator.)

Stool samples may be collected up to 3 days after the visit, if needed, and stored in a –80 °C deep freezer (performed only at Visits 2 and 4). Bowel habits—including stool frequency, stool volume, stool consistency and form (Bristol Stool Scale), and time required for defecation—will be assessed.

Based on the results, microbiome profiling and stool metabolite analyses may be conducted.

Participants will visit after a 12-hour fast on the day prior to testing, and the following laboratory tests will be performed. Remaining serum samples will be stored at –80 °C. (Only clinically necessary tests may be performed at the discretion of the principal investigator.)

① Hematology tests: RBC, WBC with differential, hemoglobin, hematocrit, and platelet count
② Blood biochemistry tests: AST, ALT, ALP, GGT, albumin, total bilirubin, direct bilirubin, total protein, uric acid, BUN, creatinine, glucose, total cholesterol, and triglycerides
③ Coagulation test: Prothrombin time (performed only at Visit 2)
④ Serum cytokine tests: TNF-α, IL-6

Participants will visit after a 12-hour fast on the day prior to testing, and the following assessments will be performed. (These tests will be conducted only for participants with fatty liver disease when clinically required as part of standard care or for those who provide consent.)
② FibroScan

A questionnaire survey will be administered (performed only at Visit 2 and Visit 4).

The above procedures will be performed only for participants who provide consent.

Contents

1. Title and Phase of the Clinical Trial

1.1 Title

A Study to Evaluate the Therapeutic Effects of Metagenomics-Based Probiotics on Non-Alcoholic Fatty Liver Disease

1.2 Phase

Investigator-Initiated Clinical Trial (IIT)

2. Name and Address of the Clinical Trial Site(s)

한림대학교춘천성심병원 / 강원도 춘천시 삭주로 7

3. Names and Titles of the Principal Investigator and Study Staff

3.1 Principal Investigator

한림대학교춘천성심병원 소화기내과 석기태 교수

3.2 Study Staff

석기태

3.3 Co-Investigators

한림대학교 춘천성심병원 소화기 내과 신석표

한림대학교 춘천성심병원 소화기 내과 양영주

한림대학교 춘천성심병원 소화기 내과 백광호

4. Name and Title of the Pharmacist Responsible for Investigational Product Management

허 연 화, 약제팀 대리

이 은 경, 약제팀 계장

오 경 미, 약제팀 계장

5. Name and Address of the Sponsor

(주)종근당바이오 / 서울특별시 서대문구 충정로 8

6. Background and Objectives of the Clinical Trial

6.1 Objectives

The objective of this study is to assess the efficacy of probiotics in improving clinical and biochemical parameters of non-alcoholic fatty liver disease (NAFLD) in diagnosed patients.

6.2 Background

-Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of conditions ranging from simple steatosis, characterized by excessive triglyceride accumulation in hepatocytes, to non-alcoholic steatohepatitis (NASH), which involves hepatocellular injury and inflammation, and ultimately to cirrhosis resulting from chronic inflammation, hepatocyte destruction, and progressive fibrosis. The pathogenesis of NAFLD is multifactorial and includes insulin resistance, lipotoxicity caused by excessive lipid accumulation, activation of Kupffer cells and hepatic stellate cells leading to inflammation and fibrogenesis, and endoplasmic reticulum stress.

- Globally, more than 600 million individuals are affected by non-alcoholic fatty liver disease (NAFLD), with the majority of cases occurring in the United States, Europe, and China. The prevalence of NAFLD continues to rise alongside increasing rates of obesity driven by unhealthy dietary habits and insufficient physical activity. In Korea, the number of patients diagnosed with NAFLD increased markedly from 24,379 in 2013 to 51,256 in 2017, representing an annual growth rate of approximately 21%. Healthcare expenditures have also escalated significantly, rising from 4.72 billion KRW in 2013 to 10.53 billion KRW in 2017, with an average annual increase of 22.7%.
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- Various pharmacologic agents have been used in attempts to treat non-alcoholic fatty liver disease (NAFLD), including anti-obesity medications (e.g., orlistat), insulin-sensitizing agents (metformin, pioglitazone, rosiglitazone), and lipid-lowering agents such as clofibrate, gemfibrozil, bezafibrate, atorvastatin, and simvastatin. Hepatoprotective agents aimed at restoring injured hepatocytes—such as ursodeoxycholic acid and taurine—along with antioxidants (vitamin E, pentoxifylline) and nutritional supplements (lecithin, betaine, N-acetylcysteine) have also been utilized. However, despite these therapeutic attempts, no pharmacologic agent has proven definitively effective for the treatment of NAFLD. Therefore, lifestyle modification, including dietary intervention, increased physical activity, and weight reduction, remains the cornerstone of current management.
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- Currently, Intercept's obeticholic acid (OCA) has demonstrated therapeutic efficacy for NASH in a phase 3 clinical trial. In addition, several pharmaceutical companies—including Genfit, Gilead, and Allergan—are conducting phase 3 trials, and numerous investigational agents with diverse mechanisms of action are in phase 2 or phase 1 development.
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- Recently, growing interest in the human microbiome has led to extensive research aimed at elucidating the relationship between microbial communities and human disease. The liver is directly connected to the gut and plays a central role in metabolizing nutrients, making the gut microbiome closely associated with liver health and disease. Increasing evidence indicates that intestinal dysbiosis and small intestinal bacterial overgrowth (SIBO) can contribute to the development and progression of liver diseases, supporting the emerging concept of the gut–liver axis.
- Lactic acid bacteria (LAB) are microorganisms that produce lactic acid through carbohydrate fermentation and have long been used to improve food preservation, flavor, nutritional value, and overall health. Beyond their established ability to modulate the gut environment, LAB have been extensively investigated for their potential therapeutic effects across various diseases. The strain ***Pediococcus pentosaceus*** KID7 used in this study is listed as an approved ingredient in the Korean Food Code and is currently incorporated in commercially available foods and dietary supplements. In addition, ***Lactobacillus helveticus*** and ***Lactobacillus bulgaricus*** are recognized as standardized probiotic ingredients under the Ministry of Food and Drug Safety (MFDS) Health Functional Food Code, allowing their use as probiotics in functional foods.
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- During the initial planning of the study, *Lactobacillus bulgaricus* CKDB001 was intended to be used instead of *Pediococcus pentosaceus* KID7. However, discrepancies were identified in the genetic analysis of *L. bulgaricus* CKDB001 compared with previous data, and additional time was required to clarify the cause of these differences. As a result, delays in strain identification and production led to the temporary replacement of the investigational strain with *P. pentosaceus* KID7. Subsequently, animal studies using *L. bulgaricus* CKDB001 yielded favorable outcomes, and this strain has been verified as suitable for use in commercially available probiotic formulations in Korea. Given its safety for human consumption and promising preclinical results, the decision was made to include *L. bulgaricus* CKDB001 in the investigational product, and the study will therefore be conducted with four arms.

- In preclinical studies using a Western diet–induced mouse model of non-alcoholic fatty liver disease (NAFLD), administration of ***Lactobacillus bulgaricus*** CKDB001 and ***Lactobacillus helveticus*** CKDB001 resulted in significant improvements, including reductions in body weight and liver weight, attenuation of hepatic steatosis and steatohepatitis, and decreased serum liver enzymes (AST and ALT). Based on these findings, the present study aims to evaluate the efficacy and safety of ***L. bulgaricus*** CKDB001 or ***L. helveticus*** CKDB001 in patients with NAFLD.
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- The marker strain of ***Pediococcus pentosaceus*** KID7 is approved as a probiotic ingredient for human consumption under the Korean Food Code and is already incorporated into commercially available formulations. Similar to the previously evaluated strains, ***P. pentosaceus*** KID7 demonstrated beneficial effects in a Western diet–induced NAFLD mouse model, including reductions in liver-to-body weight ratio, hepatic lipid accumulation, and inflammatory responses. Its administration also lowered serum liver enzymes (AST, ALT) and decreased hepatic expression of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) at both the gene and protein levels, along with a favorable modulation of the gut microbiome. These results were consistent with those observed in studies using ***L. bulgaricus*** CKDB001, providing strong justification for its inclusion as an investigational strain. Taken together, these preclinical data support the expectation that similar therapeutic benefits may be observed in patients with NAFLD.
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- The strain was initially identified in 2015 as *Lactobacillus delbrueckii* subsp. *bulgaricus* using 16S rRNA sequencing, which is one of the most commonly used methods for bacterial identification. Prior to initiating the clinical trial, whole genome sequencing (WGS) was conducted in 2019 to ensure accurate strain characterization. The WGS analysis revealed that the strain is *Lactobacillus delbrueckii* subsp. *lactis*. Accordingly, the strain name has been updated, and the study will proceed using the corrected taxonomic designation.
- The strain ***Lactobacillus delbrueckii*** subsp. ***lactis*** is listed as an approved ingredient for use in food according to the Korean Food Code, and products containing ***L. lactis*** are already commercially available as foods and health functional supplements. The strain is also certified by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) and is included in international food standards. It has been reported to lack mucin-degrading activity, biogenic amine production, hemolytic activity, and DNase activity, supporting its safety for human consumption.
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- Functionally, ***L. delbrueckii*** subsp. ***lactis*** contributes to the proliferation of beneficial probiotics, suppression of harmful bacteria, and improvement of bowel habits. In a Western diet–induced mouse model of non-alcoholic fatty liver disease (NAFLD), oral administration of ***L. lactis*** CKDB001 resulted in a reduction in liver-to-body weight ratio, decreased hepatic lipid accumulation and inflammation, reduced serum liver enzyme levels (AST, ALT), and lowered gene and protein expression of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6). Favorable modulation of the gut microbiome was also observed.

- No participants were enrolled or administered the investigational product labeled as L. bulgaricus prior to the strain name change to Lactobacillus delbrueckii subsp. lactis.

Background Summary

- Non-alcoholic fatty liver disease progresses through stages of fatty liver, steatohepatitis, and cirrhosis, and may eventually lead to hepatocellular carcinoma, resulting in significantly increased mortality.
- The high prevalence of non-alcoholic fatty liver disease (NAFLD) both in Korea and globally imposes a substantial social and economic burden.
- There is currently no approved pharmacologic therapy for non-alcoholic fatty liver disease (NAFLD).
- The gut–liver axis theory has emerged, suggesting a strong association between the gut microbiome and the development of chronic liver diseases.
- Probiotic administration is expected to improve non-alcoholic fatty liver disease (NAFLD).

7. Code Name, Active Ingredient(s), Composition, and Dosage Form of the Investigational Product

7.1 Investigational Product (IP)

7.1.1 Active Ingredient: *P. pentosaceus* KID7, *L. lactis* CKDB001 or *L. helveticus* CKDB001 (containing 9 × 10^9 CFU per day)

7.1.2 Dosage Form: Capsules

7.1.3 Strength: 300 mg

7.1.4 Storage Conditions: refrigerated condition at 4–8°C

7.2 Comparator

7.2.1 Active Ingredient: Microcrystalline Cellulose

7.2.2 Dosage Form: Capsules

7.2.3 Strength: 300 mg

7.2.4 Storage Conditions: refrigerated condition at 4–8°C

7.3 Mixing Ratio

(Mixing Ratio, %)

원료명	Investigational Product 1	Investigational Product 2	Investigational Product 3	Control Product
P. pentosaceus KID7	50	-	-	-
L. helveticus CKDB001	-	50	-	-
L. lactis CKDB001	-	-	50	
Microcrystalline Cellulose	47	47	47	97
Silicon Dioxide	2	2	2	2
Magnesium Stearate	1	1	1	1
Total	100.0	100.0	100.0	100.0

7.4 Packaging and Labeling

The investigational product will be manufactured and packaged by the sponsoring organization, Chong Kun Dang Bio Co., Ltd. (CKD Bio), and supplied to the clinical trial site through the designated research nurse. Labeling of the investigational product will comply with all applicable regulations governing investigational medicinal products and will include the required information as described below. The label will also provide space for recording the subject's allocation number and visit number to facilitate proper identification and tracking of the investigational product.

- As follows -

1. The statement "For Clinical Trial Use Only"
2. Product name or the generic name of the active ingredient
3. Expiration date
4. Storage conditions
5. Name and address of the sponsor approved for the clinical trial
6. The statement "Not for use outside the clinical trial"

7. Labeling

7.5 Management and Accountability

All investigational medicinal products will be supplied to the clinical trial site by the sponsor, Chong Kun Dang Bio Co., Ltd. The designated investigational product manager is responsible for verifying the receipt, quantity, and condition of all products delivered to the site. The manager must maintain complete records of receipt, dispensing, and return of all investigational products used in the clinical trial.

7.6 Dispensing

Dispensing of investigational medicinal products used in this clinical trial shall be conducted only upon receipt of a prescription signed by the Principal Investigator or an authorized sub-investigator participating in the study.

7.7 Storage

The investigational product manager shall record the subject's English initials, allocation number, prescription date, and quantity dispensed in the drug accountability log. The manager is responsible for storing and managing these records, monitoring the usage status of all investigational products, and maintaining complete documentation of product receipt, dispensing, and return for the clinical trial.

7.8 Return of Unused Investigational Product

The investigational product manager shall return all unused investigational medicinal products to the sponsor, Chong Kun Dang Bio Co., Ltd. If any investigational products are discarded or lost at the clinical trial site, the manager must document the event in the investigational product accountability records. At the end of the clinical trial, all unused investigational products—including those not dispensed to subjects and those returned by subjects—must be returned to the sponsor. A shipment record confirming the returned products shall be attached when sending the investigational products back to the sponsor.

8. Target Disease or Condition

Non-alcoholic fatty liver disease (NAFLD)

9. Inclusion Criteria, Exclusion Criteria, Target Sample Size, and Rationale for Sample Size

9.1 Inclusion Criteria

Subjects who meet all of the following criteria will be eligible for inclusion in the study.

1) Individuals who agree to participate in the study and have voluntarily signed the written informed consent form.
2) Male and female adults aged 20 years or older.
3) Patients diagnosed with non-alcoholic fatty liver disease (NAFLD).

※ Alcohol-related liver disease will be excluded based on the following criteria:

- Individuals with an average daily alcohol intake of ≥60 g for at least one year prior to the visit (≥30 g for women).
- *(Reference examples: 360 mL of soju at 20% alcohol ≈ 72 g of alcohol; 500 mL of beer at 5% alcohol ≈ 25 g, or 330 mL ≈ 16.5 g).*

4) Patients with elevated liver enzyme levels above the normal range.

※ (Reference Ranges for Adult Liver Enzymes

- AST (GOT): 0–30 U/L (upper limit ≤40 U/L)
- ALT (GPT): 0–38 U/L (upper limit ≤40 U/L)
- ALP: 20–130 U/L
- GGT:
  - Male: 10–62 U/L
  - Female: 7–35 U/L

9.2 Exclusion Criteria

Individuals who meet any of the following conditions will be excluded from the study.

1) Individuals who have consumed probiotics (e.g., lactic acid bacteria), prebiotics (e.g., dietary fiber, fructooligosaccharides), synbiotics, or fermented dairy products within the past 1 month.
2) Individuals who have taken antibiotics continuously within the past 2 months are expected to require antibiotic use during the study period.
3) Individuals who have continuously taken medications or health functional supplements that may affect liver function within the past 1 month.
4) Individuals who have participated in another clinical trial within the past 1 month (excluding medical device clinical trials).
5) Individuals with any of the following medical conditions:
   ① Alcoholic liver disease, hereditary metabolic liver disorders, or autoimmune hepatitis
   ② Systemic inflammatory diseases or immune-mediated disorders
   ③ Hepatocellular carcinoma
   ④ Uncontrolled cardiac or pulmonary diseases
   ⑤ Any other severe systemic disorders involving the heart, lungs, hematologic system, or endocrine system
6) Individuals with a history of malignant tumors diagnosed within the past 5 years.
7) Pregnant or breastfeeding women.
8) Individuals with known hypersensitivity or a history of severe allergic reactions to the investigational product, placebo, or any of their components.
9) Individuals deemed unsuitable for participation in the clinical trial at the discretion of the investigator.

9.3 Target Sample Size, and Rationale

(Sample size, n)

	Treatment Group 1	Treatment Group 2	Treatment Group 3	Control Group	Total
Final number of subjects for evaluation	27	27	27	27	108
Number of subjects considering a 10% drop-out rate	30	30	30	30	120

This study will be conducted in patients with non-alcoholic fatty liver disease (NAFLD) at our institution. Based on a two-group comparison in which the independent variable is binary (probiotic vs. control) and the primary outcome variable is continuous, and allowing for a 10% drop-out rate, 30 subjects per group are required.

- Null Hypothesis (H_0): There is no difference in liver function test values among the four groups, including the probiotic group and the control group.
- Alternative Hypothesis (H_1): There is a difference in liver function test values among the four groups, including the probiotic group and the control group.

Continuous Endpoint, Two Independent Sample Study
Sample Size: Group 1 27, Group 2 27

Study Parameters

Mean, group 1 50, Mean, group 2 40

Alpha 0.05 Beta 0.2 Power 0.8

[View Power Calculations]

$$k = \frac{n_2}{n_1} = 1$$
$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2/K)(z_{1-\alpha/2} + z_{1-\beta})^2}{\Delta^2}$$
$$n_1 = \frac{(15^2 + 15^2/1)(1.96 + 0.84)^2}{10^2}$$
$$n_1 = 35$$
$$n_2 = K * n_1 = 35$$

Δ = |μ2-μ1| = absolute difference between two means
σ1, σ2 = variance of mean #1 and #2
n1 = sample size for group #1
n2 = sample size for group #2
α = probability of type I error (usually 0.05)
β = probability of type II error (usually 0.2)
z = critical Z value for a given α or β
k = ratio of sample size for group #2 to group #1

※ The total number of human-derived specimens (stool samples) to be collected is 240.
((30 + 30 + 30 + 30) × 2 = 240 samples)

10. Duration of the Clinical Trial

After IRB approval –**2022.4.30**

Study Details	Study timeline							Remarks
	19.8	9-10	11-12	20.1-2	3-4	5-6	7	
IRB submission	●							
IRB approval		●	●	●				
Clinical trial		●	●	●	●	●	●	
Stool analysis					●	●	●	
Data organization and manuscript submission							●	

Study Details	Study timeline							
	8	9	10	11-12	21.01	2	3	4
IRB submission								
IRB approval								
Clinical trial	●	●	●	●	●	●	●	●
Stool analysis	●	●	●	●	●	●	●	●
Data organization and manuscript submission								

Study Details	Study timeline							
	5	6	7	8	9	10	11	12
IRB submission								
IRB approval								
Clinical trial	●	●	●	●	●	●	●	●
Stool analysis	●	●	●	●	●	●	●	●
Data organization and manuscript submission								

Study Details	Study timeline				
	22.1	2	3	4	
IRB submission					
IRB approval					
Clinical trial	●	●	●	●	
Stool analysis	●	●	●	●	
Data organization and manuscript submission			●	●	

11.Clinical Trial Methodology

11.1 Study Design

Participants who voluntarily sign the informed consent form at Visit 1 (–2 weeks) will undergo screening to determine eligibility based on the inclusion and exclusion criteria. Eligible subjects will complete a 2-week run-in period and, at Visit 2 (Week 0), will be randomly assigned—according to the order of enrollment—to one of four groups: Treatment Group 1, Treatment Group 2, Treatment Group 3, or the Control Group. Subjects will then receive the investigational product or control product for 8 weeks, after which the outcomes specified in the study assessments will be analyzed.

11.2 Dosage, Administration Method, and Duration of Intake

11.2.1 Daily Dosage and Administration Method

The clinical trial will be conducted with a total of four groups. Beginning the day after Visit 2 (Week 0), each group will receive the investigational product or control product orally three times daily for 8 weeks, taken after meals with an adequate amount of water (lukewarm water)..

Category	Morning	Afternoon	Evening
Treatment Group 1	●	●	●
Treatment Group 2	●	●	●
Treatment Group 3	●	●	●
Control Group	○	○	○

● Investigational Product (IP) 1, 2, or 3 ○ **Control Product**

11.2.2 Duration of Intake

8 weeks (56 days)

11.3 Randomization

Only subjects who meet all inclusion criteria and do not meet any exclusion criteria will be assigned a randomization number (random No.) at Visit 2 (Week 0), in the order of enrollment. The treatment allocation corresponding to each randomization number will follow a computer-generated randomization list prepared in advance by an independent third party not involved in the study.

Block randomization will be used to allocate subjects into four groups—Treatment Group 1, Treatment Group 2, Treatment Group 3, and the Control Group—in a 1:1:1:1 ratio. The gender distribution across groups will be balanced to the greatest extent possible.

As this study follows a double-blind design, the group assignment for each subject must remain concealed from both investigators and participants until completion of the clinical trial.

12. Observation Items, Clinical Laboratory Tests, Methods of Assessment, and Procedures for Sample Storage and Disposal

12.1 Study Timeline

Procedure		Screening	Baseline	Treatment	End of study
Visit		**1**	**2**	**3**	**4**
Week		**-2**	**0**[1)]	**4**	**8**
Window period (day)		**±7**		**±7**	**±7**
Informed consent		√			
Demographic characteristics		√			
Anthropometric measurements		√	√	√	√
Vital signs (pulse, blood pressure, body temperature)		√	√	√	√
Medical and surgical history		√			
Medication history		√	√	√	√
Alcohol and smoking history		√	√	√	√
Screening tests (blood and urine)	Blood tests	√			
	Pregnancy test	√			
	Viral marker testing	√			
Assessment of inclusion and exclusion criteria		√			
Randomization			√		
Administration of investigational product			√	√	
Stool sample collection and assessment of bowel habits			√		√
Clinical laboratory tests (blood)			√	√	√
Histopathological examination			√	√	√
Quality of life assessment			√		√
Liver biopsy			√		√
Assessment of adverse events		√	√	√	√

1) Visit 2 (Week 0) will be conducted 2 to 3 weeks after Visit 1 (–2 weeks). (Visit 1 and Visit 2 may be conducted on the same day if necessary.)
2) Sex, date of birth, age, menstrual status, and duration of amenorrhea (if applicable) will be recorded.
3) Body weight, height, and BMI will be measured. Height will be measured only at Visit 1.
4) Participants will undergo blood testing after a 12-hour overnight fast prior to the visit. (For participants with hepatic steatosis, the investigator may select only clinically necessary tests based on clinical judgment.)
   ① Blood chemistry tests: AST, ALT, ALP, GGT, albumin, total bilirubin, direct bilirubin, total protein, uric acid, BUN, creatinine, glucose, cholesterol, triglyceride

(These tests may be omitted for subjects who have available health examination results within the past 4 weeks.)

5) A urine pregnancy test will be performed for women of childbearing potential, except for those confirmed to be postmenopausal (amenorrhea for ≥24 months).

6) Viral marker testing will include HBsAg, Anti-HBs, and Anti-HCV. (Testing will be performed if results within the past 5 years are not available or if clinically indicated by the investigator.)

7) Stool samples may be collected up to 3 days after the visit if necessary and will be stored in a –80°C deep freezer.

Assessment of bowel habits (conducted only at Visit 2 and Visit 4)**:**

Bowel movement frequency, stool volume, stool consistency and form (Bristol Stool Scale), and time required for defecation.

8) Participants will visit after a 12-hour overnight fast for the following tests. Remaining serum samples will be stored at –80°C. (The investigator may selectively perform only clinically necessary tests based on medical judgment.)

① Hematology**:** RBC, WBC & differential count, hemoglobin, hematocrit, platelet count: RBC, WBC & differential, hemoglobin, hematocrit, platelet

② Blood chemistry: AST, ALT, ALP, GGT, albumin, total bilirubin, direct bilirubin, total protein, uric acid, BUN, creatinine, glucose, l cholesterol, triglyceride

③ Coagulation test: Prothrombin time (performed only at Visit 2)

④ Serum cytokines: TNF-α, IL-6

9) Participants will visit after a 12-hour overnight fast for the following examinations (performed only for subjects who have provided consent):

① Abdominal ultrasound or CT scan

② Fibroscan

10) Questionnaires will be administered (conducted only at Visit 2 and Visit 4).

11) Procedures will be performed only for subjects who provide consent.

12.2 Outcome measures

12.2.1 Informed consent

At Visit 1 (–2 weeks), prior to any study-related procedures, the study objectives and details will be thoroughly explained to each potential participant. Subjects who voluntarily agree to participate will sign the written informed consent form and will be assigned a Screening Number (Screening No.) in sequential order. The case report form (CRF) will document consent status, date of consent, and participant initials.

12.2.2 Demographic characteristics

At Visit 1 (–2 weeks), sex, date of birth, age, menstrual status, and duration of amenorrhea (if applicable) will be recorded.

12.2.3 Anthropometric measurements

Body weight, height, and BMI will be measured at Visit 1 (–2 weeks), Visit 2 (Week 0), Visit 3 (Week 4), and Visit 4 (Week 8). Height will be measured only at Visit 1 (–2 weeks). Measurements will be taken with shoes and outerwear removed and in the lightest clothing possible. Efforts will be made to ensure that the same equipment, time window, and study personnel are used across visits. Results will be recorded to the exact decimal places generated by the measurement device.

12.2.4 Vital signs

At each visit, pulse, blood pressure, and body temperature will be measured after the subject has rested for at least 10 minutes. Efforts will be made to use the same equipment, time window, and study personnel across visits

12.2.5 Medical and surgical history

At Visit 1 (–2 weeks), medical history will be reviewed through interviews and past medical records. Medical events within the past 5 years will be documented, including onset date (year or year/month), persistence, and investigator comments. Surgical history within the past 5 years will also be recorded, including date of diagnosis, date of discharge, and investigator comments.

12.2.6 Medication history

On each visit, use of medications, herbal medicines, and health supplements will be assessed. At Visit 1 (–2 weeks), all medications, herbal products, and supplements taken within the past 2 months will be reviewed. Any medication or supplement taken during the study will be documented, including type, dose, and duration.

12.2.7 Alcohol and smoking history

At each visit, smoking history (status, duration, amount) and alcohol intake (status, duration, amount) will be assessed.

12.2.8 Screening tests (blood and urine)

At Visit 1 (–2 weeks), after a 12-hour overnight fast, blood chemistry tests, a pregnancy test, and viral marker testing will be performed. Blood chemistry testing may be omitted for subjects with available health examination results within the past 4 weeks.
The investigator may selectively perform only clinically necessary tests.

- Pregnancy test: A urine human chorionic gonadotropin (hCG) test will be performed for women of childbearing potential, except those confirmed to be postmenopausal (amenorrhea ≥24 months).
- Viral markers: HBsAg, Anti-HBs, Anti-HCV (Performed if no results within the past 5 years or if clinically indicated.

12.2.9 Assessment of inclusion and exclusion criteria

Eligibility will be determined using data collected at Visit 1 (informed consent, demographics, anthropometry, vital signs, medical/surgical history, medication history, alcohol/smoking history, blood/urine tests), applying the inclusion and exclusion criteria described in Section 9.

12.2.10 Randomization

Eligible subjects will be randomized at Visit 2 (Week 0) into Treatment Group 1, Treatment Group 2, Treatment Group 3, or the Control Group according to the randomization procedure described in Section 11.3.

12.2.11 Administration of investigational product

Subjects will receive the assigned investigational product or control product for 8 weeks starting from Visit 2 (Week 0). All administrations will be documented.

12.2.12 Stool Sample Collection and Bowel Habit Assessment

At Visit 2 (Week 0) and Visit 4 (Week 8), stool samples will be collected. If necessary, samples may be collected within 3 days after the visit. Bowel habits will be assessed using a questionnaire, including stool frequency, stool volume, stool form (Bristol Stool Scale), and time required for defecation.

12.2.13 Clinical Laboratory Tests (Blood)

At Visit 2 (Week 0), Visit 3 (Week 4), and Visit 4 (Week 8), subjects will visit after a 12-hour overnight fast for laboratory testing.

At Visit 2, results within the past 4 weeks may be used.

① Hematology: RBC, WBC & differential, hemoglobin, hematocrit, platelet
② Blood chemistry: AST, ALT, ALP, GGT, albumin, total bilirubin, direct bilirubin, total protein, uric acid, BUN, creatinine, glucose, cholesterol, triglyceride
③ Coagulation: Prothrombin time (Visit 2 only)
④ Cytokines: TNF-α, IL-6

Clinically significant abnormal results should be documented in the CRF along with the investigator's assessment.

12.2.14 Histopathological Examinations

At Visit 2, Visit 3, and Visit 4, subjects will visit after a 12-hour fast for examinations if clinically indicated or consented.

① Abdominal ultrasound or CT: To assess the degree of hepatic steatosis
② Fibroscan: To quantitatively assess hepatic stiffness and fibrosis

12.2.15 Quality of Life Assessment

Quality of life will be assessed using a questionnaire at Visit 2 and Visit 4.

12.2.16 Liver biopsy

Liver biopsy will be performed at Visit 2 and Visit 4 only for subjects who provide consent.

12.2.17 Assessment of Adverse Events

Adverse events will be assessed at every visit.

12.2.18 Additional Visits (If Needed)

Unscheduled visits may occur if re-testing, follow-up of adverse events, or other investigator-judged needs arise.

12.3 Storage and Disposal of Collected Samples

Stool samples will be stored at –80°C and analyzed by a specialized microbiome analysis company (ChunLab).

After analysis, samples will be discarded.

Blood samples will also be stored at –80°C and discarded after analysis.

13. Expected Adverse Events and Precautions for Use

13.1Expected Adverse Events

Temporary gastrointestinal symptoms such as gas, abdominal bloating or discomfort, diarrhea, or constipation may occur after ingestion of the investigational product.

13.2Precautions for Use

1) The daily recommended intake should be followed.
2) Store the product in a cool environment (4–8°C), avoiding direct sunlight.

14. Discontinuation and Withdrawal of Study Participants

14.1 Criteria for Discontinuation and Withdrawal

1) If a serious adverse event (SAE) occurs in the participant.
2) If the participant or the participant's legal representative requests discontinuation of the study.
3) If the investigator or the participant violates the study protocol.
4) If the participant withdraws consent to participate in the study.
5) If the participant becomes lost to follow-up.
6) If the investigator determines that continued participation is not appropriate for the participant.

14.2 Management of Protocol Deviations

The principal investigator and sub-investigators must be thoroughly familiar with the study protocol and adhere strictly to its requirements to prevent protocol deviations. Study participants must follow the assigned administration instructions and comply with the study visit schedule. To support accurate dosing and adherence to scheduled visits, the investigator should take appropriate measures, such as providing written reminders of upcoming visits and conducting telephone monitoring as necessary.

In cases of **major protocol deviations**—such as violations of inclusion/exclusion criteria or failure to obtain informed consent—that may significantly affect participant safety or study outcomes, the participant should be withdrawn from the study (drop-out) as a general principle.

For **minor deviations**, the nature, extent, and reasons for the deviation must be documented accurately, and the investigator should evaluate whether the deviation may impact the study results during data analysis.

15. Criteria, Methods, and Interpretation of Efficacy Evaluation

15.1 Primary Efficacy Endpoint Analysis

After 8 weeks of administration of the investigational products or control product in the four randomized groups, the change in liver enzyme levels (AST and ALT) from baseline will be calculated for each group. Based on the results of the normality test and homogeneity of variance test, statistical comparisons between groups will be performed using either the two-sample t-test or the Mann–Whitney U test. Additional subgroup analyses may be conducted as needed.

15.2 Secondary Efficacy Endpoint Analysis

For the four randomized groups, after 8 weeks of administration of the investigational product or control product, changes from baseline will be analyzed for serum GGT, albumin, serum lipid parameters (cholesterol and triglycerides), inflammatory cytokines (TNF-α and IL-6), BMI, histopathological and imaging findings (abdominal ultrasound/CT and Fibroscan), MELD score, Child–Pugh score, worsening of liver disease (hepatic encephalopathy, ascites, variceal bleeding, spontaneous bacterial peritonitis, liver-related hospitalization, death or liver transplantation, and all-cause mortality), bowel habits (frequency of defecation, stool volume, stool consistency and form, time required for defecation), quality of life (questionnaire), and gut microbiome composition. The same statistical methods applied to the primary endpoint will be used for these analyses. Additional subgroup analyses may be conducted as needed.

15.3 Method for Stool Analysis

Among participants who provide separate written informed consent for human-derived material research, stool samples will be collected and submitted to ChunLab, a specialized bioinformatics analysis company, for microbiome analysis.

16. Criteria, Methods, and Reporting of Adverse Events and Safety Evaluation

16.1 Definitions of Terms

An *adverse event (AE)* refers to any untoward and unintended sign (including abnormal laboratory findings), symptom, or disease that occurs in a participant who has received the investigational product, regardless of whether a causal relationship with the investigational product exists.

An *adverse drug reaction (ADR)* refers to any noxious and unintended response to the investigational product that occurs at any dosage and for which a causal relationship with the investigational product cannot be ruled out.

A *serious adverse event (SAE)* refers to any adverse event occurring at any dose of the investigational product that results in any of the following outcomes:

- ✓ Death or a life-threatening condition
- ✓ Any hospitalization (initial or prolonged) (Hospitalizations solely for the purpose of conducting study procedures, hospitalizations related to the underlying NAFLD condition, or hospitalizations due to unrelated medical conditions will **not** be classified as SAEs.)
- ✓ Persistent or significant disability/incapacity
- ✓ A congenital anomaly or birth defect
- ✓ A medically important condition that may require intervention to prevent one of the outcomes listed above**.**

- As follows -

An *unexpected adverse drug reaction* refers to a reaction whose nature or severity is not consistent with the available product information, such as the investigator's brochure or the product's labeling/insert.

16.2 Safety Evaluation Population

Safety evaluations will be conducted for all participants who have taken the investigational product at least once.

16.3 Criteria and Methods for Adverse Event Evaluation

Adverse events will be evaluated by considering the frequency and severity of events documented in each participant's adverse event log, as well as abnormalities identified in laboratory tests (hematology, blood chemistry, urinalysis) and vital signs. Clinically significant abnormalities in laboratory results or vital signs will be recorded in the adverse event section of the case report form (CRF).
For each adverse event, the following information will be documented in the CRF: occurrence of the event, signs and symptoms, date of onset and resolution, severity, relationship to the investigational product, actions taken regarding the study drug, treatments administered, and clinical outcome.

16.4 Severity of Adverse Events

The severity of adverse events will be classified according to their maximal intensity based on the following criteria.

No.	Criteria	내용
1	Mild, Grade 1	Does not interfere with the participant's normal daily activities, causes minimal discomfort, and is easily tolerated.
2	Moderate, Grade 2	Causes noticeable discomfort and interferes with the participant's normal daily activities or functioning.
3	Severe, Grade 3	Prevents the participant from performing normal daily activities or functioning.

16.5 Relationship to the investigational product

The investigator will assess the relationship between the investigational product and each adverse event by reviewing the participant's medical history, current health status, timing of administration, dosing conditions, and other relevant factors, and will determine causality according to the following criteria.

No.	Criteria	Content
1	Certain	✓ There is clear evidence that the investigational product was administered ✓ The temporal relationship between administration of the investigational product and the onset of the adverse event is clinically plausible ✓ The adverse event is more reasonably explained by the investigational product than by other causes ✓ The adverse event resolves or improves after discontinuation of the investigational product (positive dechallenge) ✓ The adverse event recurs upon re-administration of the investigational product, if rechallenge is performed (positive rechallenge) ✓ The nature of the adverse event is consistent with known information regarding the investigational product or other agents in the same class
2	Probable/likely	✓ There is clear evidence that the investigational product was administered ✓ The temporal relationship between administration of the investigational product and the onset of the adverse event is clinically plausible ✓ The adverse event is more plausibly explained by the investigational product than by other causes ✓ The adverse event resolves or improves after discontinuation of the investigational product (positive dechallenge)
3	Possible	✓ There is clear evidence that the investigational product was administered ✓ The temporal relationship between administration of the investigational product and the onset of the adverse event is clinically plausible ✓ The investigational product is considered as likely a cause of the adverse event as other plausible etiologies ✓ The adverse event resolves or improves after discontinuation of the investigational product (positive dechallenge)
4	Unlikely	✓ There is clear evidence that the investigational product was administered. ✓ There are other causes that are more likely to explain the adverse event. ✓ The adverse event does not resolve, or the findings are inconclusive, after discontinuation of the investigational product (negative or equivocal dechallenge). ✓ The adverse event does not recur, or the findings are inconclusive, after re-administration of the investigational product if performed (negative or equivocal rechallenge)
5	None	✓ The participant did not receive the investigational product; or ✓ The temporal relationship between drug administration and the onset of the adverse event is not plausible; or ✓ There is a clear alternative explanation for the adverse event.
6	Unassessable	✓ There is partial information regarding the adverse event, but the available data are insufficient to determine its relationship to the investigational product.

16.6 Actions taken with respect to the investigational product

1) Dose increased
2) Dose not changed
3) Dose reduced
4) Temporarily discontinued
5) Permanently discontinued
6) Not applicable
7) Unknown

16.7 Medication for Adverse Events

1) Medication administered for the adverse event
2) No medication administered for the adverse event

16.8 Outcome of Adverse Event

1) Recovered (resolved)
2) Recovering (resolving)
3) Not recovered (not resolved)
4) Recovered with sequelae
5) Fatal
6) Unknown

16.9 Methods for Reporting Adverse Events

The responsibilities of each party regarding any serious adverse event (SAE) that occurs during the clinical trial are as follows.

16.9.1 Responsibilities of the Principal Investigator

If a serious adverse drug reaction (SADR) occurs during the clinical trial, the principal investigator must report it to the sponsor immediately, and no later than 1 business day from the date the investigator becomes aware of the event. A follow-up report containing detailed information shall also be submitted subsequently.
In the case of an unexpected serious adverse drug reaction (USADR), the investigator must promptly report the event to both the sponsor and the Institutional Review Board (IRB).
If a fatal case is reported, the principal investigator shall provide the sponsor and the IRB with additional documentation, such as the autopsy report (if performed) and the death certificate or other relevant medical documentation.

16.9.2 Responsibilities of the Sub-Investigator

If a serious adverse event related to the investigational product occurs during the clinical trial, the sub-investigator (study staff) must immediately report the event to the principal investigator and the sponsor, and subsequently submit a follow-up report containing detailed information.
In the case of an unexpected serious adverse drug reaction (USADR), the sub-investigator must promptly report the event to both the sponsor and the Institutional Review Board (IRB).

16.9.3 Responsibilities of the Institutional Review Board (IRB)

If an unexpected serious adverse drug reaction or any new information arises that may negatively affect participant safety or the conduct of the clinical trial, the Institutional Review Board (IRB) shall require the principal investigator to take appropriate actions as necessary.

16.9.4 Responsibilities of the Sponsor

The sponsor shall report all serious and unexpected adverse drug reactions (SADRs/USADRs) to the relevant investigators, and when necessary, to the Institutional Review Board (IRB) and the Ministry of Food and Drug Safety (MFDS), within 15 days from the date the sponsor receives notification from the principal investigator or sub-investigator, or from the date the sponsor otherwise becomes aware of the event.
In cases where the adverse reaction results in death or is life-threatening, the sponsor shall report the event within 7 days of becoming aware of it and shall submit a follow-up report with detailed information within 8 days of the initial report. When submitting an adverse drug reaction report, the sponsor shall include the information provided by the principal investigator or sub-investigator.

The sponsor must provide additional safety information periodically until the adverse drug reaction is considered resolved (i.e., the reaction has subsided, or further follow-up is not possible). In such cases, the investigator shall actively cooperate by providing all necessary data and supporting information required for ongoing reporting.

16.10 Pregnancy Reporting Procedures

Pregnancy itself is not considered an adverse event during the clinical trial. In addition, hospitalization for elective termination without complications (excluding therapeutic abortion) or for normal delivery of a healthy newborn is not considered an adverse event.

Once a pregnancy is identified, the investigator must complete an Initial Pregnancy Report and notify the sponsor within 24 hours of becoming aware of the pregnancy. The investigator shall follow and document the course and outcome of all pregnancies, even if the participant withdraws consent or discontinues participation in the clinical trial.

Furthermore, the investigator must complete a Pregnancy Outcome Report and notify the sponsor within 24 hours after becoming aware of the outcome of the pregnancy.

17. Participant Information Sheet and Informed Consent Form

The Participant Information Sheet and Informed Consent Form (ICF) may be used only after approval by the Institutional Review Board (IRB). The investigator must obtain informed consent from each participant in accordance with the ethical principles of the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines.

Prior to performing any procedures related to this clinical trial, the investigator shall fully explain the study to the participant (or the participant's legally authorized representative) and obtain written informed consent. The investigator must retain the signed original copy of the consent form in the investigator's file, and provide the participant (or legally authorized representative) with a signed copy of the consent form along with the information sheet that was explained.

If any revisions are made to the Participant Information Sheet or the Informed Consent Form, the revised documents must receive **prior re-approval** from the IRB. Participants who are currently enrolled in the study (or their legally authorized representatives) must also undergo **re-consent** using the approved revised version. In such cases, the investigator must document the individuals notified, the date and time of notification, and the content delivered as source documentation.

18. Compensation for Injury

In the event that an adverse reaction caused by the investigational product, or an injury arising from medical interventions performed to manage such adverse reactions, results in harm to a participant, the participant will be compensated in accordance with the **Compensation for Injury Policy**, provided that the injury is directly attributable to the investigational product.

19. Post-Trial Medical Care and Treatment Guidelines

Participants who discontinue the clinical trial prematurely, as well as those who complete the study, will receive subsequent medical care and treatment according to the standard clinical practices and established guidelines for patients with liver disease.

20. Safety Protection Measures for Study Participants

20.1 Clinical Trial Institution

The head of the clinical trial institution shall ensure that the necessary clinical trial facilities, equipment, and qualified personnel required for each stage of the study are fully secured and properly prepared so that the clinical trial can be conducted appropriately and safely.

20.2 Principal Investigator

The principal investigator shall be fully familiar with the adverse events, precautions, and safety information described in this clinical trial protocol. If any serious adverse event occurs during the trial, the principal investigator must immediately report it to the Institutional Review Board (IRB) and the sponsor.

20.3 Sub-Investigator

The sub-investigator shall be fully familiar with the expected adverse events and precautions specified in this protocol. If a serious adverse event occurs during the conduct of the clinical trial, the sub-investigator must immediately report it to the principal investigator and the sponsor.

21. Other Requirements for the Safe and Scientific Conduct of the Clinical Trial

21.1 Compliance with GCP and the Declaration of Helsinki

The procedures described in this protocol have been developed to ensure that the investigator and the sponsor conduct, evaluate, and document the clinical trial in compliance with ICH-GCP, the Korean Good Clinical Practice (KGCP) guidelines, and the ethical principles outlined in the Declaration of Helsinki. This clinical trial will be conducted in accordance with all applicable domestic laws and regulatory requirements.

21.2 Informed Consent

The investigator must thoroughly explain all relevant aspects of the clinical trial to the participant and/or the participant's legally authorized representative, providing sufficient opportunity to ask questions and understand all foreseeable implications of participation. All informed consent must be documented in writing.
The investigator must sign the informed consent form to confirm that the consent process was properly conducted. No procedures performed solely for the purposes of the clinical trial may be initiated until informed consent has been obtained from the participant.

21.3 Approval of the Clinical Trial Protocol

Before initiating the clinical trial, the protocol and all related documents must be submitted to the Institutional Review Board (IRB) in accordance with applicable regulations. The clinical trial may begin only after IRB approval has been obtained.

21.4 Confidentiality

All participant names will remain confidential, and participants will be identified in records and evaluations only by the subject identification number assigned within the clinical trial. Participants will be informed that all clinical trial data will be stored in a secure computer system and handled strictly confidentially. When transferring clinical trial data from the clinical trial site, all personally identifiable information (including name, address, or any direct identifiers) must be removed, and only the subject identification code may be transmitted.
Signed informed consent forms will be retained by the principal investigator. By signing this protocol, the principal investigator agrees to obtain informed consent appropriately from each participant and to allow audits or inspections upon request. The principal investigator must maintain a list linking subject names to subject identification numbers, which must be kept securely for record verification.
The informed consent forms and subject identification list shall be stored in a designated archiving room for **three years** from the product approval date (or three years from study completion if the product is not intended for approval). After this period, the documents may be disposed of.

21.5 Familiarization with the Clinical Trial Protocol

The principal investigator and all study staff must thoroughly review, understand, and adhere to the clinical trial protocol prior to conducting the study.

21.6 Clinical Trial Monitoring and Audits

The sponsor shall conduct a pre-study visit before trial initiation. To ensure that the clinical trial is conducted in compliance with KGCP and that the clinical trial data can be accepted for domestic or international regulatory submission, the sponsor will conduct monitoring and audits throughout the trial.
During monitoring visits, the monitor will verify that case report forms (CRFs) are complete and accurate and compare CRF entries with source documents in the presence of the investigator or designated study staff. The investigator must always cooperate fully with the sponsor.

IRB Confirmation Letter (English)
Confirmation of Delayed Patient Enrollment

IRB No.	2019-08-005	Date	January 12, 2026
Study Title	Clinical study for the efficacy of probiotics in the improvement of non-alcoholic fatty liver disease		
Principal Investigator Affiliation	Hallym University Chuncheon Sacred Heart Hospital, Division of Gastroenterology	Principal Investigator Name	Prof. KITAE SUK
Description	This letter is issued to confirm the timeline and circumstances of patient enrollment for the following clinical study. This study is a Clinical study for the efficacy of probiotics in the improvement of non-alcoholic fatty liver diseaseudy, and the first patient was enrolled after September 20, 2020. The Institutional Review Board (IRB) has reviewed the Informed Consent Form and Participant Information Sheet of the first enrolled subject and confirms that the patient was properly consented using IRB-approved documents prior to enrollment. The IRB further confirms that the delay in patient recruitment compared with the originally planned schedule was due to restrictions related to the COVID-19 pandemic and delays in research funding execution.		

This confirmation is issued to officially certify the above facts.

Institutional Review Board, Hallym University Chuncheon Sacred Heart Hospital